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L1: Entry 1 of 1

File: USPT

Feb 27, 2001

US-PAT-NO: 6194548DOCUMENT-IDENTIFIER: US 6194548 B1

TITLE: Green fluorescent proteins and blue fluorescent proteins

DATE-ISSUED: February 27, 2001

INVENTOR-INFORMATION:

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FOREIGN-APPL-PRIORITY-DATA:

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JP	10-026418	January 23, 1998

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US-CL-ISSUED: 530/350; 435/440, 536/23.1, 935/10

US-CL-CURRENT: 530/350; 435/440, 536/23.1

FIELD-OF-SEARCH: 530/350, 435/440, 536/23.1, 935/10

PRIOR-ART-DISCLOSED:

U.S. PATENT DOCUMENTS

☐ Search Selected☐ Search ALL

PAT-NO	ISSUE-DATE	PATENTEE-NAME	US-CL
<input type="checkbox"/> <u>5491084</u>	February 1996	Chalfie et al.	435/189
<input type="checkbox"/> <u>5625048</u>	April 1997	Tsien et al.	536/23.4
<input type="checkbox"/> <u>5777079</u>	July 1998	Tsien et al.	530/350

FOREIGN PATENT DOCUMENTS

FOREIGN-PAT-NO	PUBN-DATE	COUNTRY	US-CL
95/21191	August 1995	WOX	
96/27675	September 1996	WOX	
97/11094	March 1997	WOX	
97/42320	November 1997	WOX	

OTHER PUBLICATIONS

Chen, C. et al., "High-efficiency Transformation of Mammalian Cells by Plasmid DNA," Molecular and Cellular Biology, 7: 2745-2752 (1997).
Heim, R. et al., "Wavelength Mutations and Posttranslational Autoxidation of Green Fluorescent Protein," Proc. Natl. Acad. Sci. USA, 91: 12501-12504 (1994).
Heim, R. et al., "Engineering green fluorescent protein for improved brightness, . . ." Current Biology, 6: 178-182 (1996).
Siemering, K., "Mutations that suppress the thermosensitivity . . ." Current Biology, 6: 1653-1663 (1996).
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Yang et al. (Apr. 3, 1998) J.Biol. Chem., vol. 273, pp. 8212-8216.

ART-UNIT: 162

PRIMARY-EXAMINER: Achutamurthy; Ponnathapu

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ABSTRACT:

This invention relates to novel fluorescent GFPs and BFPs. A novel BFP according to this invention has an F64L mutation as well as a L236R mutation and is provided with improved fluorescence. Furthermore, another BFP has the F64L mutation with the characteristics as described above and other mutations, V163A and S175G, and it possesses markedly improved characteristics in the expression at 37.degree. C. in addition to those as described above.

27 Claims, 7 Drawing figures

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Tsukamoto; Noriyo	Hyogo			JPX
Yamasaki; Masatoshi	Hyogo			JPX

US-CL-CURRENT: 530/350; 435/440, 536/23.1

CLAIMS:

What is claimed is:

1. A fluorescent protein comprising the amino acid sequence set forth in SEQ ID No. 1 in the Sequence Listing, said sequence comprising at least mutations of Phe64Leu, Val163Ala, and Ser175Gly.
2. A fluorescent protein comprising the amino acid sequence set forth in SEQ ID No. 1 in the Sequence Listing, said sequence consisting of the three mutations of Phe64Leu, Val163Ala, and Ser175Gly.
3. A fluorescent protein comprising the amino acid sequence set forth in SEQ ID No. 1 in the Sequence Listing, said sequence comprising at least mutations of Tyr66His, Tyr145Phe, Phe64Leu, and Leu236Arg.
4. A fluorescent protein comprising the amino acid sequence set forth in SEQ ID No. 1 in the Sequence Listing, said sequence consisting of the four mutations of Tyr66His, Tyr145Phe, Phe64Leu, and Leu236Arg.
5. A fluorescent protein comprising the amino acid sequence set forth in SEQ ID No. 1 in the Sequence Listing, said sequence comprising at least mutations of Tyr66His, Tyr145Phe, Phe64Leu, Val163Ala, Ser175Gly and Leu236Arg.
6. A fluorescent protein comprising the amino acid sequence set forth in SEQ ID No. 1 in the Sequence Listing, said sequence consisting of the six mutations of Tyr66His, Tyr145Phe, Phe64Leu, Val163Ala, Ser175Gly and Leu236Arg.
7. A fluorescent protein of SEQ ID NO:1 comprising at least mutations of Tyr66His, Tyr145Phe, Val163Ala and Ser175Gly.
8. A fluorescent protein of SEQ ID NO:1 consisting of mutations of Tyr66His, Tyr145Phe, Val163Ala and Ser175Gly.
9. A fluorescent protein comprising the amino acid sequence set forth in SEQ ID No. 1 in the Sequence Listing, said sequence consisting of mutations of Tyr66His, Tyr145Phe, and Phe64Leu.
10. The protein of claim 1 further comprising one or more mutations selected from the group consisting of Ser65Thr, His231Leu and a valine inserted between Met1 and Ser2.
11. The protein of claim 3 further comprising one or more mutations selected from the group consisting of Ser65Thr, His231Leu and a valine inserted between Met1 and Ser2.
12. The protein of claim 5 further comprising one or more mutations selected from the group consisting of Ser65Thr, His231Leu and a valine inserted between Met1

and Ser2.

13. The protein of claim 7 comprising one or more mutations selected from the group consisting of Ser65Thr, His231Leu and a valine inserted between Met1 and Ser2.

14. A fluorescent protein comprising the amino acid sequence set forth in SEQ ID No. 1 in the Sequence Listing, said sequence consisting of mutations of Phe64Leu, Val163Ala, and Ser175Gly and one or more mutations selected from the group consisting of Ser65Thr, His231Leu and a valine inserted between Met1 and Ser2.

15. A fluorescent protein comprising the amino acid sequence set forth in SEQ ID No. 1 in the Sequence Listing, said sequence consisting of mutations of Tyr66His, Tyr145Phe, Phe64Leu, and Leu236Arg and one or more mutations selected from the group consisting of Ser65Thr, His231Leu and a valine inserted between Met1 and Ser2.

16. A fluorescent protein comprising the amino acid sequence set forth in SEQ ID No. 1 in the Sequence Listing, said sequence consisting of mutations of Tyr66His, Tyr145Phe, Phe64Leu, Val163Ala, Ser175Gly and Leu236Arg and one or more mutations selected from the group consisting of Ser65Thr, His231Leu and a valine inserted between Met1 and Ser2.

17. A fluorescent protein comprising the amino acid sequence set forth in SEQ ID No. 1 in the Sequence Listing, said sequence consisting of mutations of Tyr66His, Tyr145Phe, Val163Ala and Ser175Gly and one or more mutations selected from the group consisting of Ser65Thr, His231Leu and a valine inserted between Met1 and Ser2.

18. A fluorescent protein comprising the amino acid sequence set forth in SEQ ID No. 1 in the Sequence Listing, said sequence consisting of mutations of Tyr66His, Tyr145Phe, and Phe64Leu and one or more mutations selected from the group consisting of Ser65Thr, His231Leu and a valine inserted between Met1 and Ser2.

19. The protein of claim 1, further comprising one or more mutations selected from the group consisting of His231Leu and a valine inserted between Met1 and Ser2.

20. The protein of claim 3, further comprising one or more mutations selected from the group consisting of His231Leu and a valine inserted between Met1 and Ser2.

21. The protein of claim 5, further comprising one or more mutations selected from the group consisting of His231Leu and a valine inserted between Met1 and Ser2.

22. The protein of claim 7, further comprising one or more mutations selected from the group consisting of His231Leu and a valine inserted between Met1 and Ser2.

23. The protein of claim 14, wherein said one or more mutations selected from the group consisting of His231Leu and a valine inserted between Met1 and Ser2.

24. The protein of claim 15, wherein said one or more mutations selected from the group consisting of His231Leu and a valine inserted between Met1 and Ser2.

25. The protein of claim 16, wherein said one or more mutations selected from the group consisting of His231Leu and a valine inserted between Met1 and Ser2.

26. The protein of claim 17, wherein said one or more mutations selected from the group consisting of His231Leu and a valine inserted between Met1 and Ser2.

27. The protein of claim 18, wherein said one or more mutations selected from the group consisting of His231Leu and a valine inserted between Met1 and Ser2.